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CURRENT LITERATURE

NOTES FOR STUDENTS

Metabolism of fungi.—Recently a number of papers on the metabolism of fungi have appeared, which, although they represent various phases of the subject, may be noted here in a collective review. Since PASTEUR'S discovery of the biological method for separating stereoisomeric components from their racemic compounds, the study of the action of fungi on compounds having asymetric carbon atoms has been of great interest. The work of the earlier investigators, like that of LE BELL, LEUKOWITSCH, SCHULZE and BOSSHARD, and others, was concerned chiefly with the chemical aspects of the subject, with the purpose of resolving racemic compounds into their optically active components.

Taking up the subject more in its biological aspect, for the purpose of determining whether any fungi are able to utilize both components of racemic compounds to an equal extent, PRINGSHEIM[1] has investigated the action of 16 fungi and 2 bacteria on leucine and glutaminic acid, from which SCHULZE and BOSSHARD[2] had obtained *d*-leucine and *l*-glutaminic acid by the action of *Penicillium*. PRINGSHEIM found that in all cases both of the components of the amino-acids used were partly consumed by the organisms. In about one-half of the experiments both components were consumed to an equal degree, so that the recovered portions of the acids were optically inactive. In the remaining instances one isomer was consumed to a greater extent than the other, the naturally occurring component being the one consumed most readily in all such cases.

HERZOG and his students have taken up the study of the action of fungi on *d-l*-oxyacids and *d-l*-amino-acids in order to gain a knowledge of the process involved in the utilization of one of the isomers of the inactive forms of these acids. The experimentation was carried out both with living fungi and with mycelia killed by various means. In the experiments with living material the fungi were grown in flasks of suitable culture media until the carbon dioxide production became constant. A definite quantity of the acid to be tested was then introduced into the flasks and the subsequent carbon dioxide output determined. At the end of the experiment the residual acid was determined and its rotation measured. In the experiments with fungi killed by acetone, methyl alcohol, or other means, the powdered fungus material was added to flasks containing solutions of the acids. The carbon dioxide products and residual acid were determined as before.

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[1] PRINGSHEIM, HANS, Studien über Spaltung racemischer Aminosäuren durch Pilze. Zeitschr. Physiol. Chem. **65**:96–109. 1910.

[2] SCHULZE, E., und BOSSHARD, E., Untersuchungen über die Aminosäuren welche bei der Zersetzung der Eiweissstoffe durch Salzsäure und durch Barytwasser entstehen. II. Zeitschr. Physiol. Chem. **10**:134–145. 1886.

In the first experiments reported by HERZOG and MEIER[3] it was found that the addition of lactic, tartaric, malic, mandelic, and β-oxybutyric acids to cultures of *Penicillium glaucum*, in which the carbon dioxide production had become fairly constant, resulted in a great increase of the carbon dioxide output. In every case the excess of carbon dioxide over the normal was much greater than that calculated on the assumption that all of the acid used up had been completely oxidized to carbon dioxide and water. Under the same conditions glycollic, citric, pyrotartaric, and oxybutyric acids gave no increase in the carbon dioxide production. The authors suggest that these experiments indicate that the biological splitting of substances containing an asymmetric carbon atom depends on a process of oxidation. Only the acids having an asymmetric carbon atom were oxidized.

To test this hypothesis further, experiments were carried out with fungous material killed with acetone and methyl alcohol and finely pulverized. Definite quantities of the material were added to flasks containing solutions of lactic acid or sodium lactate, and also to control flasks containing distilled water. It was found that the carbon dioxide production in the flasks containing the acid or its salt was slightly greater than in the controls.

In a second paper[4] the method of experimentation with killed mycelia is applied to the study of a number of other acids. The mycelia in these experiments were immersed in liquid air, by which, it was assumed, the cells were killed, although the spores were subsequently found to be alive. In these experiments it was found that *d*-tartaric acid, *l*-tartaric acid, and *d-l*-tartaric acid were oxidized, while mesotartaric acid, which is not separable into optically active components, was left intact. The dextro-rotatory form was oxidized most rapidly. The optically active isomers of lactic acid showed scarcely any difference in the rate of oxidation, while *l*-mandelic acid was oxidized more rapidly than its antipode. Glycollic acid, having no asymmetric carbon atom, was not attacked. The authors conclude that the preferential oxidation of one component of a racemic mixture, which has heretofore been regarded as biological selections of food substances, is merely the result of differences in the reaction velocities of the antipodes with the substances of the organisms.

In continuation of the foregoing work, HERZOG and RIPKE[5] have studied the effects of *Oidium lactis* killed with acetone and ether on the lactic, succinic, and mandelic acids. The results obtained do not conform with those obtained in the foregoing experiments with *Penicillium.* Only lactic acid gave a greater carbon dioxide production than the control with distilled water. With mandelic and succinic acids the control flasks yielded greater amounts of carbon dioxide. In an experiment in which the fungus was left in liquid air for several hours, subsequent cultures showed that the cells had not all been killed.

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[3] HERZOG, R. O., und MEIER, A., Ueber Oxydation durch Schimmelpilze. Zeitschr. Physiol. Chem. **57**:35–42. 1908; also MEIER, A., Dissertation under the same title. Karlsruhe. 1909.

[4] ———. *Ibid.* **59**:57–62. 1909.

[5] HERZOG, R. O., und RIPKE, O., Ueber das Verhalten einger Pilze zu organischen Säuren. *Ibid.* **73**:284–289. 1911.

A similar set of experiments carried out by HERZOG, RIPKE, and SALADIN[6] with acetone preparations of *Mycoderma cerevisiae* showed that with acetic acid and lactic acid the carbon dioxide production was less in the acid medium than in distilled water, although a part of the acid in each case had disappeared. The carbon dioxide output in experiments with the different isomeric modifications of mandelic and tartaric acids was not determined, but the whole added quantity of these acids could not be recovered. In some cases with mandelic acid the total acid content of the controls at the end of the experiment was as great as that in flasks to which acid had been added. The authors assume that the autolytic production of acid by the killed fungus cells reaches a certain maximum. If that maximum has been attained by the addition of a foreign acid, no further spontaneous acid formation occurs. Succinic acid depressed the production of carbon dioxide, but there was no evidence that any of the acid had disappeared. The general conclusion from this last set of experiments is that the production of carbon dioxide by killed cells of *Mycoderma* is depressed in acid media, although the quantity of acid is diminished. The disappearance of the acid, therefore, cannot be explained as a process of oxidation, nor is the process one of metabolism, since the cells were dead. In view of the comparatively small quantities of acids which disappeared apparently through the action of the killed fungus cells, the experiments would have been more convincing if the authors had reported control experiments showing how much of the acids could be immediately recovered from the mixtures.

In another paper by HERZOG and SALADIN[7] the effect of leucine on the carbon dioxide production of *Penicillium* is reported. The method of experimentation was similar to that described above in the experiments of HERZOG and MEIER, and the results were comparable to those obtained with oxyacids. The addition of leucine was followed by an increased production of carbon dioxide, which was greater than that calculated on the assumption that all the available leucine had been oxidized to carbon dioxide and water.

The important series of researches of EHRLICH[8] on the behavior of amino-acids in alcoholic fermentation has greatly advanced our knowledge of the origin of fusel-oils, which have usually been regarded as side-products of sugar fermentation. These investigations have been extended by EHRLICH and JACOBSON[9] to other fungi, to determine whether the decomposition of amino-acids induced by them is similar to that brought about by the yeast cell. The authors have studied the action, on amino-acids, of some 50 fungi, upon which a complete report is promised. The behavior of the filamentous fungi toward amino-acids differs greatly according to whether carbohydrates are present or not. In the absence of carbohydrates the decomposition of amino-acids is more extensive than that produced by yeasts, but in the presence of carbohydrates the degree of decomposition differs with different fungi. In the present paper the peculiar behavior of *Oidium lactis* on amino-acids in the presence of sugar is reported. The action of this fungus results in the replacement of the amino-groups by hydroxyl, thereby yielding oxyacids corresponding to the amino-acids according to the following general reaction:

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[6] HERZOG, R. O., RIPKE, O., und SALADIN, O. *Ibid.* **73**: 290–301. 1911.

[7] HERZOG, R. O., und SALADIN, O., Ueber das Verhalten einiger Pilze gegen Aminosäuren. *Ibid.* **73**: 302–307. 1911.

[8] For a general account of this work see EHRLICH, F., Ueber die chemischen Vorgänge pflanzlichen Erweissstoffwechsels und ihre Bedeutung für die Alkoholische Gärung und andere pflanzenphysiologische Processe. Landwirth. Jahrb. **38**: 289–327. 1909.

$$R \cdot CH(NH_2)CO_2H + H_2O = R \cdot CH(OH)CO_2H + NH_3$$

The ammonia which is formed is used in protein synthesis by the fungus. Here as with the yeast cell the amino-nitrogen (in the form of ammonia) enters into the metabolism of the cell, while the rest of the molecule is excreted as a product not capable of being further utilized. The end products in the two cases are different, being in the case of the yeast cell an alcohol with one carbon less than the amino-acid from which it was derived, while with *Oidium lactis* an oxyacid corresponding to the amino-acid results. By the action of *Oidium lactis*, *l*-tyrosin yielded *d*-paraoxyphenyl-lactic acid, *d-l*-phenylalanin yielded *d*-phenyl-lactic acid, and *l*-tryptophan gave *l*-indol-lactic acid, all acids which were heretofore not known in those modifications.

In this connection the authors point out that KOTAKE[10] obtained from dogs suffering from phosphorus poisoning the levorotatory form of oxyphenyl-lactic acid, thus affording an example of the production by the plant and by the animal cell, not of the same but of opposite stereoisomers from one and the same substance. It should be stated, however, that KOTAKE himself regards it as extremely improbable that his acid was produced from tyrosin, as he was unable to isolate oxyphenyl-lactic acid as a result of feeding tyrosin itself.

A better example of the production of isomeric antipodes from the same racemic substance is afforded by the action of plant cells and of animal cells on racemic phenylamino-acetic acid. NEUBAUER and WARBURG[11] obtained the

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[9] EHRLICH, FELIX, und JACOBSON, K. A., Ueber die Umwandlung von Aminosäuren in Oxysäuren durch Schimmelpilze. Ber. Deutsch. Chem. Gesells. **44**: 888–897. 1911.

[10] KOTAKE, Y., Ueber *l*-Oxyphenylmilchsäure und ihr Vorkommen im Harn bei Phosphorvergiftung. Zeitschr. Physiol. Chem. **65**: 397–401. 1910.

[11] NEUBAUER, O., und WARBURG, O., Ueber eine Synthese mit Essigsäure in der künstlich durch bluteten Leber. *Ibid.* **70**: 1–9. 1910.

d-acetylphenylamino-acetic acid from this substance injected into the liver of dogs, while NEUBAUER and FROMHERZ[12] obtained the *l*-acetylphenylamino-acetic acid as a result of yeast fermentation of the same racemic compound.

The intermediate chemical transformations by which *d*-amino-acids are changed into alcohols with one carbon atom less in fermentation have been investigated by NEUBAUER and FROMHERZ and described in the paper cited above. The content of the paper is largely chemical. The conclusion is reached that the amino-acids are not directly transformed into alcohols by hydrolysis and subsequent splitting off of ammonia and carbon dioxide, as represented by the general formula

$$R\cdot CH\cdot NH_2-COOH+H_2O=R\cdot CH_2OH+CO_2+NH_3$$

but that keto-acids are first formed, and these, by loss of carbon dioxide and reduction, are changed into alcohols, the main steps of the process being represented as follows:

$$\begin{aligned}&R-CHNH_2-COOH+O=R-CO-COOH+NH_3\\&R-CO-COOH\rightarrow R\cdot CHO+CO_2\\&R-CHO+2H=R-CH_2OH\end{aligned}$$

This interpretation is the result of experiments in which it was shown (1) that a keto-acid (phenylglyoxylic acid) was formed by the fermentation of phenylamino-acetic acid, and (2) that a keto-acid (*p*-oxyphenylpyrotartaric acid) yielded the same alcohol by fermentation as the corresponding amino-acid (tyrosin).[13] A number of side reactions and secondary products occur in this process. The occurrence of the aldehyde is postulated. According to the authors the decomposition of amino-acids by yeasts is hereby shown to follow the same course as the decomposition of these acids in the animal body, except that the postulated aldehyde which is reduced to alcohol by the yeast cell is oxidized to the corresponding fatty acid which is further utilized in metabolism by the animal cell.

The discovery by EHRLICH[14] of the production of fumaric acid from sugar through the agency of *Rhizopus nigricans* is of great biological interest, not only because it is the first instance of the occurrence of fumaric acid as a product of metabolism of micro-organisms, but also because of its possible bearing on the origin of unsaturated acids in higher plants. The acid was isolated by EHRLICH from culture solutions, containing much sugar, upon which *Rhizopus nigricans* was grown. The quantity of acid varies with the sugar content, but in old cultures from which the sugar has disappeared the acid is again consumed.

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[12] NEUBAUER, O., und FROMHERZ, K., Ueber den Abbau der Aminosäuren bei der Hefegärung. Zeitschr. Physiol. Chem. **70**: 326–350. 1911.

[13] EHRLICH, FELIX, Ueber die Vergärung des Tyrosins zu *p*-Oxyphenyl-äthylalkohol (Tyrosol). Ber. Deutsch. Chem. Gesells. **44**: 139–146. 1911.

[14] ———, Ueber die Bildung von Fumarsäure durch Schimmelpilze. Ber. Deutsch. Chem. Gesells. **44**: 3737–3742. 1911.

With glycerin, alcohol, or peptone as sources of carbon, no fumaric acid is produced. The fact that fumaric acid here occurs as an intermediate product in the metabolism of sugar suggests that unsaturated acids in higher plants may result from carbohydrate metabolism. That a close relation exists between the higher unsaturated acids and carbohydrates in plant metabolism has been generally conceded by plant physiologists since the work of MAQUENNE.

That many fungi and bacteria are able to utilize fats has been shown by several investigators. A further contribution to the subject has been made by OHTA,[15] who studied the decomposition of the fat of horse liver caused by five forms of fungi obtained by exposing culture plates in the laboratory. The forms were *Cladosporium herbarum*, *Penicillium glaucum*, *Aspergillus glaucus*, *A. nidulans*, and *Actinomucor repens*. All of these caused the disappearance of fat from the ground sterilized liver tissue. *Actinomucor* was the most active, causing the disappearance of over 60 per cent of the fat in three weeks. Of the others, *Aspergillus* used 17–20 per cent, *Cladosporium* 14 per cent, and *Penicillium* 6–8 per cent. Attempts to grow *Actinomucor* on culture solutions containing fat as the only source of carbon, in order to study the mode of decomposition of the fat, were unsuccessful. The paper contains detailed notes on the methods and precautions to be observed in making fat determinations in work of this kind.

Another contribution to the subject of the utilization of fat by fungi has been made by ROUSSY,[16] who experimented with the following forms: *Absidia glauca*, *Circinella umbellata*, *Mucor mucedo*, *Phycomyces nitens*, *Rhizopus nigricans*, *Sporodinia grandis*, *Morteirella candelabrum*, *Aspergillus flavus*, *Citromyces glaber*, *Penicillium luteum*, *Sterigmatocystis nigra*, and *Sporotrichum bombyceum*. All of these grew well on fats and oils of various kinds. To determine if it was the fatty acid or the glycerine which was utilized, cultures were made in Raulins solution in combination with oleic, palmitic, or stearic acid or glycerine. It was found that the fungi thrived well on the fatty acids, but only *Aspergillus* and *Penicillium* grew on glycerin solutions.

REICHEL, studying the effects of acetic acid and its salts on a form of *Penicillium*, has rediscovered the fact that the toxicity of that acid is mainly due to the action of the undissociated molecule. He finds that acetic acid is poisonous in much lower concentrations than those at which the strong mineral acids are toxic, but, owing to its slight dissociation, its toxicity cannot be attributed to the hydrogen ion. At the same time, the salts of acetic acid, which are highly dissociated, are not poisonous, hence the acetate ion is not poisonous. The toxicity of acetic acid, therefore, must be attributed to the molecule as a whole.

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[15] OHTA, KOHSHI, Ueber die fettzehrenden Wirkungen der Schimmelpilze nebst dem Verhalten des Organfettes gegen Fäulniss. Biochem. Zeitschr. **31**: 177–194. 1911.

[16] ROUSSY, A., Sur la vie des Champignons dans les acides gras. Compt. Rend. **153**: 884–886. 1911.

The same conclusion reached by CLARK[17] by similar experimentation and reasoning many years ago seems to have escaped his notice.

REICHEL[18] further points out that the addition of mineral acids to solutions containing acetates produces the same effect as the addition of acetic acid, since the acetic acid is replaced in its salts by the stronger acids and undissociated acid is formed in the solution as a result of the establishment of a new equilibrium. In solutions whose acidity is not great enough to inhibit growth entirely, the author finds a certain regulatory depression of the acidity by the fungus until more favorable concentrations are attained. This phase of the subject seems to demand further investigation to determine whether such purposeful regulation really exists. Under certain conditions, at any rate, depending upon the substances available in the medium, either acid or alkali will accumulate in culture solutions through the action of fungi to such a degree as to inhibit growth entirely.[19]

BORKORNY[20] reports a number of miscellaneous experiments and observations indicating that methyl alcohol can be used as a source of carbon by some fungi and bacteria. A yeast not capable of fermenting cane sugar or glucose grew spontaneously on a solution of mineral nutrients to which 0.1 per cent of methyl alcohol had been added. Inoculations from this culture were made in solutions containing 0.0025 per cent to 5 per cent methyl alcohol. After a time the flasks contained vegetations of yeast and bacteria and in some cases infusoria were present. Apparently no precautions were taken to avoid contamination, so that it is possible that carbon compounds were introduced in the form of dust particles.

SAITO[21] reports the formation of lactic acid by *Rhizopus chinensis*, thus confirming the observations of EIJKMAN and of CHRZASZCZ, who reported the production of lactic acid by *Rhizopus Rouxii*. These observations had been doubted because other instances of the production of lactic acid by filamentous fungi are not known. SAITO identified his acid by means of the zinc and the calcium salts and by the reaction of UFFELMAN.

GOUPIL[22] finds that *Rhizopus Rouxii* produces in cultures up to 4 grams per liter of succinic acid mixed with some acetic and some butyric acid. Contrary to the statements of some workers, oxalic and lactic acids are not produced. He believes that the succinic acid is formed from sugar and not from any amino-acid.—H. HASSELBRING.

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[17] CLARK, J. F., On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. BOT. GAZ. **28**: 289–327; 378–404. 1899.

[18] REICHEL, J., Ueber das Verhalten von *Penicillium* gegenüber der Essigsäure und ihren Salzen. Biochem. Zeitschr. **30**: 152–159. 1910.

[19] HASSELBRING, H., The carbon assimilation of *Penicillium*. BOT. GAZ. **45**: 176–193. 1908.

[20] BORKORNY, TH., Beobachtungen über Pilze, welche Methylalkohol als C-Quelle verwenden können. Centralbl. Bkt. II. **29**: 176–188. 1911.

[21] SAITO, K., Ein Beispiel von Milchsäurebildung durch Schimmelpilze. Centralbl. Bakt. II. **29**: 289–290. 1911.

[22] GOUPIL, R., Recherches sur *l'Amylomyces Rouxii*. Compt. Rend. **153**: 1172–1174. 1911.

Current taxonomic literature.—G. E. OSTERHOUT (Muhlenbergia **8**:44, 45. 1912) characterizes a new *Cogswellia* (*C. concinna*) and a new variety of *Gnaphalium* (*G. decurrens* var. *glandulosum*) from Colorado.—O. PAULSEN (Arb. Bot. Have Kb. no. 65. 303–318. 1911) under the title "Marine plankton from the East-Greenland Sea" records the Peridiniales found on the Danish Expedition to Greenland in 1906–1908 and describes a new species of *Peridinium* (*P. varicans*), also a new species doubtfully referred to *Apodinium*. —W. H. RANKIN (Phytopathology **2**:28–31. *pl. 3.* 1912) describes and illustrates a new fungus (*Sclerotinia Panacis*) which is said to be the cause of a root-rot of ginseng; it was found near Apulia, N.Y.—A. B. RENDLE, E. G. BAKER, S. MOORE, and A. GEPP (Journ. Linn. Soc. Bot. **40**:1–245. *pls. 1–7.* 1911) have published an important paper entitled "A contribution to our knowledge of the Flora of Gazaland." The paper includes a general descriptive account of the country concerned, records about 1000 species of which approximately 180 are new to science. The plants were collected by Mr. C. F. M. SWYNNERTON and the types are deposited in the herbarium of the British Museum.—R. A. ROLFE (Bot. Mag. *t. 8417.* 1912) describes and illustrates a new species of *Stanhopea* (*S. peruviana*) from Peru, and (Kew Bull. 131–135. 1912) has published several new species of orchids including 4 from Panama and South America.—E. ROSENSTOCK (Rep. Sp. Nov. **10**:274–280. 1912) under the title "Filices costaricenses" has published 11 new species of ferns.—P. A. RYDBERG (Torreya **12**:1–11. 1912) in continuation of studies of the plants collected on the Peary arctic expeditions gives a list of the plants secured by Drs. WOLF and GOODSELL; the article includes a new species of *Conioselinum* (*C. pumilum* Rose) from Labrador. The same author (Bull. Torr. Bot. Club **39**:99–111. 1912) under "Studies on the Rocky Mountain flora XXVI" describes a new species in *Deschampsia* and one in *Anticlea*. Two new generic names are proposed, namely *Hesperochloa*, based on *Poa* (?) *Kingii* Wats., and *Dipterostemon*, based on *Brodiaea capitata* Benth.—C. S. SARGENT (Rep. Mo. Bot. Gard. **22**:67–83. 1911) under the heading "Crataegus in Missouri II" has described 14 new species.—A. K. SCHINDLER (Rep. Sp. Nov. **10**:403, 404. 1912) has published a new genus (*Kummerowia*) based on *Hedysarum striatum* Thunb. a species common to the New and Old World.—H. SCHINZ (Vierteljahrsschrift Naturf. Gesells. Zürich **56**:229–268. 1911) in an article "Beiträge zur Kenntnis der afrikanischen Flora" has proposed the following new genera belonging to the Amarantaceae: *Centemopsis*, *Nelsia*, *Neocentema*, and *Lopriorea*.—R. SCHLECTER (Rep. Sp. Nov. **10**:248–254, 291–296, 352–363, 385–397, 445–461. 1911–1912) under the title "Orchidaceae novae et criticae" has published about 70 new species of orchids from Central and